

The University of Chicago

A STUDY OF THE REACTIONS OF  
ERGOSTEROL WITH MERCURIC ACETATE  
AND WITH ANHYDROUS AURIC  
CHLORIDE

A DISSERTATION SUBMITTED TO THE FACULTY  
OF THE DIVISION OF THE PHYSICAL SCIENCES  
IN CANDIDACY FOR THE DEGREE OF DOCTOR  
OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

1935

By  
LEONA VIVIAN IOB

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## INTRODUCTION

The importance of various types of unsaturation in molecules in intermediary metabolism is a subject to which increased attention is being devoted. Among these compounds, ergosterol, pro-vitamin D, has been put to various experimentation in an attempt to elucidate the effect of ultra-violet light upon the molecule. Ultra-violet light may bring about the following changes in an unsaturated molecule: (a) it may cause polymerization; (b) it may cause the rupture of a carbon to carbon bond, as in the case of the photochemical conversion<sup>1</sup> of cyclohexanone into 1-hexenal; or (c) it may produce an electromer by the intermolecular displacement of electrons within the molecule. From the latter standpoint, the antirachitic vitamin may be an electromer of ordinary ergosterol. To test this hypothesis, a color reaction, characteristic of ergosterol, was developed, and various isomers and derivatives of ergosterol, and compounds of similar structure were tested both before and after irradiation. Another reaction, characteristic of ergosterol, that of dehydrogenation of the molecule by the use of mercuric acetate, was investigated, and the results served as the basis for deductions concerning the structure of ergosterol.

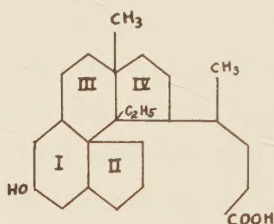
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<sup>1</sup> Ciamician and Silber, Ber., 41, 1071 (1908).

## PREVIOUS WORK

### The New Cholane Formula

During the past three years the conception of the constitution of the sterols and the bile acids has undergone drastic changes. Formerly the bile acid, cholic acid, was written:



Wieland 1928

In this formula, the fusion of three rings about a quarternary carbon atom at position C<sub>9</sub> results in an unusual ring system. Three such rings cannot be in plane configuration. X-ray examination<sup>1</sup> of the sterols pointed clearly to the fact that the accepted formulae for these compounds could not be made to fit into the crystallographic cells. The dimensions of the ergosterol molecule and the position of the side chain in the accepted sterol formula could not be reconciled with the experimentally determined length of the molecule.

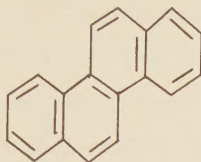
In conformity with the requirements of the results of x-ray crystallography, Rosenheim and King<sup>2</sup> introduced a

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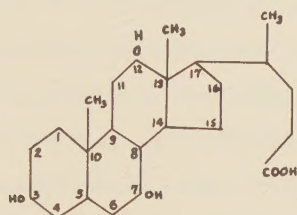
<sup>1</sup>Bernal, J. Soc. Chem. Ind., 51, 466 (1932).

<sup>2</sup>Rosenheim and King, J. Soc. Chem. Ind., 51, 464 (1932).

fundamentally new structure for these cholane substances, based upon the phenanthrene structure of chrysene,  $C_{18}H_{12}$ ,



which Diels isolated from among the dehydrogenation products of cholesterol. This formula, for cholic acid,



Rosenheim and King, 1932

Wieland and Dane, 1932

was at once accepted by Windaus.<sup>1</sup>

The new cholane formula, based primarily upon the formation of chrysene in selenium dehydrogenation of cholesterol,<sup>2</sup> consists essentially of a reduced phenanthrene ring system to which ring III of the old formula is attached to a cyclopentane ring, which carries the side chain at the position  $C_{17}$  in the molecule instead of  $C_{19}$ . This formula satisfies the requirements of the x-ray measurements of Bernal,<sup>3</sup> and is in accord with the experimental facts as shown by Windaus. This revolutionary change in the concept of the cholane formula had a still further application in the elucidation of the ring system of the oestrogenic

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<sup>1</sup>Windaus, Z. physiol. Chem., 213, 147 (1932).

<sup>2</sup>Diels, Gädke, and KÖrding, Ann., 459, 1 (1927); Ber., 60, 140 (1927).

<sup>3</sup>Bernal, Nature, 129, 277 (1932).



hormones. Thus the apparently unrelated series of physiologically highly active substances, as crystalline vitamin D, oestrogenic hormones, carcinogenic hydrocarbons, and cardiac glucosides, are shown to have the phenanthrene nucleus in common.<sup>1</sup>

The side chain in cholic acid is allocated to C<sub>17</sub>. This hypothesis offers a natural explanation for the ease with which ring closure occurs between the side chain and ring III in the derivatives of deoxycholic acids with the carbonyl group at C<sub>12</sub>, and satisfies the x-ray requirements.

The position of the two tertiary methyl groups at C<sub>10</sub> and C<sub>13</sub> is not definitely proved. The presence of a methyl group at C<sub>10</sub> follows from the isolation of  $\alpha$ -methyl glutaric acid, CO<sub>2</sub>H.CHCH<sub>3</sub>.CH<sub>2</sub>.CH<sub>2</sub>.CO<sub>2</sub>H, from a diketo-dicarboxylic acid, and from a step-wise degradation of ring I of cholesterol<sup>2</sup> to a monobasic acid which presumably has a tertiary methyl group adjacent to the carboxyl. This assumption is based upon the fact that the acid is very difficult to esterify. Furthermore, the observation of Wieland,<sup>3</sup> that the dicarboxylic acid, C<sub>22</sub>H<sub>38</sub>O<sub>4</sub>, resulting from the ultimate degradation of rings I and II in cholesterol forms an anhydride and not a cyclic ketone is compatible with the assumption of a methyl group at C<sub>10</sub>. The position of the second methyl is allocated to C<sub>13</sub>. This assumption is supported by the fact that Diel's hydrocarbon, C<sub>18</sub>H<sub>16</sub>, obtained by selenium dehydrogenation of cholic acid, has been identified as 3-methylpentenophenanthrene.<sup>4</sup>

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<sup>1</sup>Rosenheim and King, Ann. Rev. Biochem., 3, 87 (1934).

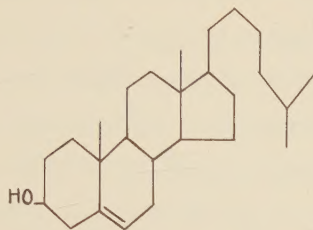
<sup>2</sup>Tschesche, Ann., 498, 185 (1932).

<sup>3</sup>Wieland and Dane, Z. physiol. Chem., 216, 91 (1933).

<sup>4</sup>Kon, J. Soc. Chem. Ind., 52, 950 (1933).

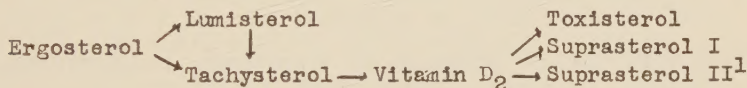


Upon this basis the following formula for cholesterol is presented:



The knowledge obtained from the elucidation of the structure of cholesterol is at once applicable to the problem of the constitution of ergosterol. The structure of ergosterol is however uncertain to a degree that makes impossible, except as conjecture, the formulation of the complex changes occurring in the production of the vitamin by the action of ultra-violet light.

Ergosterol,  $C_{28}H_{44}O$ , a sterol containing three double bonds, a secondary alcohol group, may be transformed completely into other products as a result of irradiation by ultra-violet light.



Five of the six products as thus formulated have been isolated in crystalline form either as the free alcohols or as the esters. Toxisterol alone has not been isolated, but its existence is based upon measurements of absorption spectra and upon the toxicity of the toxisterol fraction of the irradiated products. Irradiation of ergosterol produces the following changes: a lower melting point, an increase in solubility, a shift in optical rotation from  $-132^{\circ}$  to  $+15^{\circ}$ , and a shift towards general absorption in the

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<sup>1</sup>Lettre, Ann., 511, 280 (1934).

ultra-violet spectrum. The chief band of the antirachitic compound occurs at 265 to 270  $m\mu$ . Irradiated ergosterol does not yield a precipitate with digitonin. It contains an hydroxyl group as indicated by the formation of an urethane upon treatment with phenylisocyanate. It loses its antirachitic power when heated in vacuo at 180° for 4 hours, and is not readily attacked by oxygen. Windaus<sup>1</sup> believes that in the photochemical transformation of ergosterol into vitamin D, the molecular formula, the hydroxyl group, and the three double bonds remain unchanged, and that all that occurs is a steric or structural rearrangement which leads to an increase in the spacial size of the molecule.

#### Knowledge of the Structure of Ergosterol

The side chain of ergosterol is known to be  $\text{.CH(CH}_3\text{).CH:CH.CH(CH}_3\text{).CH(CH}_3\text{)}_2$  with the double bond occurring between  $C_{22}$  and  $C_{23}$ . This was demonstrated by Guiteras<sup>2</sup> and Reindel and Kipphan,<sup>3</sup> who isolated 1-isopropylmethylacetaldehyde by the action of ozone upon ergosterol.

The hydroxyl group is allocated to ring I as a result of Reindel's work<sup>4</sup> on the oxidation of ergostanol (hexahydroergosterol) to a dicarboxylic acid,  $C_{28}H_{48}O_4$ , which gives a pyroketone. The position of the hydroxyl group in ring I is usually allocated to  $C_3$  by analogy with cholesterol, but the observation of Heilbron, Lamant, and Simpson<sup>5</sup> that chloroergostane (the hydroxyl group of ergostanol replaced by chlorine) on oxidation gives a chloronor-cholanolic acid different from the known 3-chloroallonorcholanolic

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<sup>1</sup>Windaus, Proc. Roy. Soc. (London) B., 108, 568 (1931).

<sup>2</sup>Guiteras, Nakamija, and Inhoffen, Ann., 494, 116 (1932).

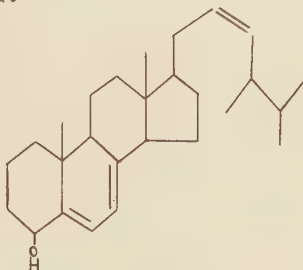
<sup>3</sup>Reindel and Kipphan, Ann., 493, 181 (1932).

<sup>4</sup>Reindel, Ann., 466, 131 (1928).

<sup>5</sup>Heilbron, Lamant, and Simpson, J. Chem. Soc., 1933, 302.

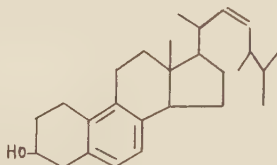
acid prepared from cholesterol causes considerable doubt as to the correctness of this position.

From the reaction of ergosterol with maleic anhydride at  $135^{\circ}$  to give an addition compound, and from the intense ultra-violet absorption of ergosterol, a conjugated system of double bonds is postulated, and the following provisional formula for ergosterol is suggested:



#### Some Reactions of the Labile Ergosterol Molecule

Ergosterol is converted into a pinacone  $C_{56}H_{84}O_2$  by the action of light in the presence of a photosensitizer. Upon pyrolysis or upon heating the pinacone in decalin solution, methane is evolved, and a new sterol, neoergosterol,<sup>1</sup> is formed. The ring containing the two double bonds becomes aromatic. Honigmann,<sup>2</sup> by dehydrogenation by the use of platinum, isolated the picrate of a phenol. Since a phenol can be formed only as a result of the production of an aromatic ring in ring I, Honigmann concludes that the original nuclear double bonds of neoergosterol must be in ring II and neoergosterol is represented by the following formula:



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<sup>1</sup>Inhoffen, Ann., 497, 130 (1932).

<sup>2</sup>Honigmann, Ann., 511, 298 (1934).



The easy dehydrogenation of neoergosterol is made possible by the absence of the tertiary methyl group at C<sub>10</sub>.

Ergosterol reacts with selenium dioxide,<sup>1</sup> and with mercuric acetate,<sup>2</sup> producing not the expected keto derivative, but a dehydrogenation product, dehydroergosterol, which contains four double bonds.

The double bond in the side chain of ergosterol is not involved in the characteristic reactions of the molecule.<sup>3</sup> The sterol produced by the hydrogenation of ergosteryl acetate maleic anhydride, 22-dihydroergosterol, shows all the characteristic reactions of ergosterol. It is as laevorotatory as ergosterol, shows the characteristic absorption spectra of ergosterol between 250 and 310 m $\mu$ , and gives a maleic anhydride addition compound. In the absence of oxygen, 22-dihydroergosterol forms a pinacone in the presence of eosin and light. This pinacone upon heating decomposes with the loss of methane into 22-dihydroneoergosterol. Irradiation of 22-dihydroergosterol with ultra-violet light produces a product active antirachitically in doses of 0.5 to 1.0 . The compound is 30 - 40 times weaker than vitamin D<sub>2</sub>. The chemical reactions of ergosterol and 22-dihydroergosterol are similar, except that 22-dihydroergosterol does not give methylisopropyl-acetaldehyde upon ozonization.

#### The Irradiation Products of Ergosterol

That the process of isomerization of ergosterol by ultra-violet light has occurred in several stages is evident from the isolation of five well-characterized isomerides of ergosterol. Of these isomerides, calciferol, or vitamin D<sub>2</sub>, alone possesses

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<sup>1</sup>Callow and Rosenheim, J. Chem. Soc., 1933, 387.

<sup>2</sup>Windaus and Linsert, Ann., 465, 157 (1928).

<sup>3</sup>Windaus and Langer, Ann., 508, 105 (1933-1934).

antirachitic activity. The suprasterols are products of over-irradiation. Since ergosterol alone is precipitable by digitonin, epimerization of the hydroxyl group is thought to have taken place at the lumisterol stage. In lumisterol, tachysterol, and calciferol,<sup>1</sup> a nuclear system of conjugated double bonds is still present, but in what manner it differs from ergosterol is not known. In the suprasterols, the conjugated system is absent. In all cases the position of the double bond in the side chain is unaltered.

Complete hydrogenation of vitamin D<sub>2</sub>, lumisterol, and suprasterol I and II gives four different hexahydro-derivatives, which also differ from the hexahydro-derivatives of ergosterol, ergostanol, epi-ergostanol, and  $\gamma$ -ergostanol.<sup>2</sup> In the ergosterol formula with 10 asymmetric centers, there are  $2^{10}$  or 1024 possible optical isomers. Thus it is not surprising that ultra-violet light produces more than one isomer of ergosterol. The derivatives of ergosterol, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, D, F, and G, produced by chemical means, give ergostanol upon hydrogenation.

Ergosterol is oxidized by concentrated nitric acid to methylbenzenetetracarboxylic acid, a fact which leads to the conclusion that a dihydrobenzene system exists in the molecule. Of the irradiated products of ergosterol, lumisterol alone is converted into the same acid.<sup>3</sup>

Selenium dehydrogenation of ergosterol under certain conditions produces the fully aromatic hydrocarbon, chrysene, C<sub>18</sub>H<sub>12</sub>. Selenium dehydrogenation of vitamin D<sub>2</sub> produces no characteristic hydrocarbon per se, nor any substance which yields a crystalline picrate. Seemingly the structure of the carbon skeleton of

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<sup>1</sup>Lettre', Ann., 511, 280 (1934).

<sup>2</sup>Ahrens, Fernholz, and Stoll, Ann., 500, 109 (1933).

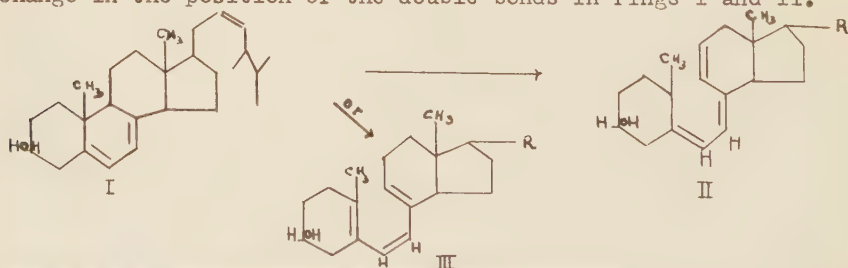
<sup>3</sup>Inhoffen, Ann., 494, 124 (1932).

vitamin D<sub>2</sub> is different from that of the isomeric ergosterol.

Of all the irradiation products of ergosterol, tachysterol differs most markedly from the parent substance. The absorption at 280 m $\mu$  is more than double that of ergosterol.<sup>1</sup> The product is extraordinarily sensitive to air, forms a peroxide in the absence of a sensitizer. It adds citraconic anhydride quantitatively at room temperature, whereas ergosterol reacts with maleic anhydride only at 135°.<sup>2</sup>

Tachysterol is easily changed by heating to 190°. Ergosterol may be heated to 200° without change. Experiments upon the degree of unsaturation<sup>3</sup> of tachysterol suggest that the molecule contains four double bonds. Perbenzoic acid titration of the dinitrotolyl ester and of the completely hydrogenated citraconic anhydride addition product of tachysterol indicates that tachysterol agrees in behavior with a tetra-unsaturated dehydroergosterol. This finding, that tachysterol has four double bonds, three of which apparently conjugated, explains the marked reactivity of the molecule with citraconic anhydride and the ease of autoxidation.

Lettre offers as an explanation of this photochemical reaction which changes ergosterol into tachysterol a rupture of a carbon to carbon linkage. A new double bond is thus created in the molecule as a result of the migration of hydrogen atoms, or a change in the position of the double bonds in rings I and II.



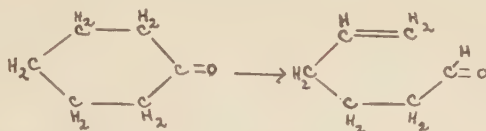
<sup>1</sup>Windaus, v. Werder, and Lüttringhaus, Ann., 499, 188 (1932).

<sup>2</sup>Windaus and Lüttringhaus, Ber., 64, 850 (1931).

<sup>3</sup>Lettre, Ann., 511, 280 (1934).



An analogous photochemical reaction<sup>1</sup> is cited:



in which the C-C bond of the ring is broken, and the migration of a hydrogen atom produces a double bond. The manner in which tachysterol is changed into vitamin D<sub>2</sub> is still an open question. The negative findings in the selenium dehydrogenation and the fact that tachysterol, probable precursor of vitamin D<sub>2</sub>, is structurally different from ergosterol strongly suggests that vitamin D<sub>2</sub> also differs from ergosterol.

The infra-red absorption spectrum<sup>2</sup> of vitamin D<sub>2</sub> differs from that of ergosterol, and also suggests a change in the structure of the vitamin molecule.

The crude irradiation product of ergosterol when heated shows an interesting behavior. It loses its antirachitic potency, but retains its toxicity. Upon further irradiation, however, the product regains its antirachitic activity, although to a much smaller degree. Busse<sup>3</sup> has shown that pure vitamin D<sub>2</sub> loses both antirachitic potency and toxicity upon heating, and cannot be re-activated by renewed irradiation. The presence of lumisterol and tachysterol is responsible for the characteristic behavior of the crude irradiation product of ergosterol, not the presence of vitamin D<sub>2</sub>.

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<sup>1</sup>Ciamiciam and Silber, Ber., 41, 1071 (1908).

<sup>2</sup>Husch and Kellner, Biochem. Z., 235, 162 (1931).

<sup>3</sup>Busse, Z. physiol. Chem., 214, 211 (1933).

## PLAN OF PROCEDURE

The plan of attack of this sterol study included three distinct aspects: (1) the activation of ergosterol without the use of ultra-violet light; (2) the detection of minute quantities of ergosterol; (3) the investigation of the characteristic behavior of the conjugated system in the ergosterol molecule.

### I. The Activation of Ergosterol without the Use of Ultra-Violet Light

Numerous experiments upon the isomerization of ergosterol have not as yet led to any method whereby vitamin D can be obtained other than by the irradiation of ergosterol by ultra-violet light. Many chemical reagents were used for the purpose of activating ergosterol without the use of ultra-violet light. A thorough study was made of the effect of antioxidants, peroxides, polymerizing agents, traces of metals, and other reagents upon the activation of ergosterol by ultra-violet light. Ergosterol and quinone, phenanthroline, and rosindone, et cetera, were irradiated with visible light and tested for biological activity. Suffice it to say, no chemical compound was discovered which activated ergosterol even by use of visible light.

### II. The Detection of Minute Quantities of Ergosterol: The Reaction of Ergosterol with Anhydrous Auric Chloride

The direct action of anhydrous auric chloride upon aromatic substances leads to the introduction of the gold into the aromatic nucleus and the formation of a compound  $RAuX_2$ . Kharasch and

Isbell's<sup>1</sup> results indicate that direct "auration" is as general a reaction as nitraction, halogenation, or mercuration, except that it is a much faster reaction and the products of the reaction are much less stable.

Ergosterol in anhydrous, thiophene-free benzene and anhydrous auric chloride<sup>2</sup> in nitrobenzene give a beautiful, characteristic rose pink color. In concentrated solution, ergosterol reacts with auric chloride, liberating free gold; the reaction is perhaps similar to that of mercuric acetate<sup>3</sup> or selenium dioxide,<sup>4</sup> and the resulting compound is dehydroergosterol. In dilute solutions, when the auric chloride is dissolved in a nonreactive solvent as nitrobenzene, a rose pink color is given to the nitrobenzene solution by a very few crystals of ergosterol. If benzene is substituted for nitrobenzene, and merely a trace of auric chloride in nitrobenzene is added, the color is more intense and fades more slowly.

#### Range of Detection and Duration of the Color

A standard solution of ergosterol, 10 mgm. per litre of benzene, was prepared. This solution when diluted 1:10 with benzene produces with the auric chloride reagent a pink color which endures 45 seconds: thus 0.001 mgm. of ergosterol is detectable. The standard solution (0.01 mgm.) produces a pink color which fades entirely within 2 minutes. It was found that if benzonitrile were substituted for nitrobenzene as solvent for the auric chloride, much less decomposition of the auric chloride into aurous chloride takes place as a result of the formation of the more

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<sup>1</sup>Kharasch and Isbell, J. Am. Chem. Soc., 53, 3053 (1931).

<sup>2</sup>Beck, Ph. D. Thesis, University of Chicago (1932).

<sup>3</sup>Windaus and Linser, Ann., 465, 157 (1928).

<sup>4</sup>Callow and Rosenheim, J. Chem. Soc., 1933, 387.

stable cyanophenyl auric dichloride. If merely a trace of the cyanophenyl auric dichloride is added to a very dilute solution of ergosterol, 0.005 mgm. or less, the pink color developed is stronger in intensity and fades less rapidly.

#### The Nature of the Color Complex

The color is discharged by additions of unsaturated compounds, i.e., heptene or amylene, and by compounds containing oxygen, examples of which are ethers, alcohols and aldehydes of the aliphatic series. The color is sensitive to changes in temperatures. The color of the standard ergosterol solution was maintained for two hours at  $-80^{\circ}$ .

Cod-liver oil, diluted with benzene, produces a deep blue color with the anhydrous auric chloride reagent. This color distribution, pink with ergosterol and blue with cod-liver oil, is precisely the same as that of the Carr-Price reaction of anti-mony trichloride with ergosterol and carotin. A sample of pure carotin, in dilutions comparable to those of ergosterol (0.001 mgm.) produces the same blue color as shown by the diluted cod-liver oil. The residue of an ether extract of raw carrot gives the blue coloration.

If the rose color developed by the interaction of ergosterol with anhydrous auric chloride is due to compound formation of the ergosterol with auric chloride and not to the formation of colloidal gold, such a rose colored solution, whose color has been discharged by the addition of an unsaturated compound as heptene, should show no Tyndall effect, due to the light-scattering by the particles of colloidal gold. The rose colored solution of the ergosterol observed in the chamber of a Leitz slit ultra-microscope showed no light scattering. A similar ergosterol-auric chloride solution, whose color had been discharged by the addition



of amylene, likewise showed no light-scattering, and was therefore free of colloidal particles.

When, however, Caritol, a commercial product of carotin in oil, was diluted with benzene and treated with the auric chloride reagent, and this solution was run through the chamber of the microscope, some light-scattering was observed. When the color of a fresh solution of Caritol and auric chloride was discharged by the addition of amylene, and the colorless solution run through the chamber of the microscope, the same light-scattering was seen. Whether Caritol reduces auric chloride to colloidal gold or whether the original oil base of the Caritol solution was responsible for the light-scattering was not determined.

This color reaction was tested with various derivatives and isomers of ergosterol, and with their irradiation products. The results are listed in Table I.

TABLE I  
COMPOUNDS TESTED WITH ANHYDROUS AURIC CHLORIDE  
ERGOSTEROL DERIVATIVES AND ISOMERS

| Compound                                                 | Color Produced |
|----------------------------------------------------------|----------------|
| 1. Ergosterol                                            | Rose Pink      |
| 2. Ergosteryl $\alpha$ -acetate*                         | " "            |
| 3. Ergosteryl $\beta$ -acetate*                          | " "            |
| 4. Ergosteryl benzoate                                   | " "            |
| 5. Ergosteryl phenyl urethane*                           | " "            |
| 6. Ergosteryl methyl ether*<br>(Methyl oxyergostatriene) | " "            |
| 7. HBr isomer of ergosterol**                            | No Color       |
| 8. HCl isomer of ergosterol**                            | " "            |
| 9. Dehydroergosterol                                     | " "            |

\*After prolonged irradiation, these derivatives show the same rapid fading of the auric chloride color as does ergosterol.

\*\*Isomers of ergosterol resulting from a shift in the position of the conjugated double bonds under the influence of mineral acids, HCl and HBr.

TABLE II

COMPOUNDS TESTED WITH ANHYDROUS PLATINIC CHLORIDE

| Compound                        | Color Produced |
|---------------------------------|----------------|
| 1. Carotin                      | No Color       |
| 2. Ergosterol                   | Pink           |
| 3. Ergosteryl benzoate          | Faint Pink     |
| 4. Ergosteryl $\alpha$ -acetate | Pink           |
| 5. Ergosteryl $\beta$ -acetate  | "              |
| 6. Methyl ergosteryl ether      | "              |

The Reaction of Irradiated Ergosterol  
with Auric Chloride

The irradiation of ergosterol in ether for one hour at the distance of 6 inches from an hanging, air-cooled, quartz, mercury vapor ultra-violet lamp appears to produce a solution which gives a fainter pink color when treated with anhydrous auric chloride. Actually, irradiation for 20 minutes converts about one-fourth of the ergosterol into vitamin D<sub>2</sub>. Vitamin D<sub>2</sub> may not show this reaction with auric chloride. Solutions of Viosterol in oil 250 D showed no formation of color. All attempts to prepare vitamin D<sub>2</sub> solutions by irradiation and by the precipitation of the unchanged ergosterol by the addition of digitonin, even after hydrolysis of the soluble digitonide, resulted in solutions which gave no color test with anhydrous auric chloride. These solutions, however, were found to be very active biologically, when fed to rachitic animals, and therefore contained the active principle.

Solutions of ergosterol in benzene were irradiated for five hours. Solutions containing 1 mgm. per cc. showed a far more rapid fading of the auric chloride color than the unirradiated standard solution containing 0.01 mgm. per cc. This almost instantaneous fading of the auric chloride color of 1 mgm. solutions of ergosterol irradiated for long periods is considered to indicate increased

reducing power of the ergosterol molecule. This solution showed antirachitic activity in doses 100 times the curative dose (100%).

Ergosterol solutions in benzene were evacuated to  $10^{-4}$  mm. pressure by means of a vacuum line and a three-stage mercury vapor pump, sealed, and irradiated by the rays of the quartz mercury vapor lamp for 8 hours. A similar tube was irradiated in air for the same length of time. The solution of the evacuated tube was tested with auric chloride reagent, and in dilutions of 1 mgm. ergosterol per cc. gave a faint yellowish pink that rapidly reduced the auric chloride to gold. When treated in a similar manner, the solution from the unsealed tube gave no color, or an instantaneous pink which was very difficult to detect. The solution from the evacuated tube alone showed any antirachitic activity in doses of 10%. Both solutions were 100 times more concentrated than the un-irradiated standard solution which gives a pronounced pure rose pink color which persists for some time. The reducing properties of the molecule have been greatly increased by prolonged irradiation, and these properties are independent of the presence of oxygen. This increased reducing power of ergosterol resulting from ultra-violet irradiation may be explained by Honigmann's findings that tachysterol,<sup>1</sup> and vitamin D<sub>2</sub>, by analogy, contain four double bonds instead of the three of the parent substance.

Many unsaturated substances, compounds containing conjugated systems of double bonds, and several terpenes were tested with the anhydrous auric chloride reagent. The results, with the exception of 1,3 dibromobutene 2, were negative as shown by Table III.

The ergosteryl acetates,  $\alpha$  and  $\beta$ , ergosteryl benzoate, ergosteryl phenyl urethane, methoxyergostatriene, the HCl and the

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<sup>1</sup>Honigmann, loc. cit.

TABLE III

UNSATURATED COMPOUNDS TESTED WITH ANHYDROUS  $\text{AuCl}_3$  REAGENT

| Compound                                                 | Color Developed                                        |
|----------------------------------------------------------|--------------------------------------------------------|
| 1. Ergosterol                                            | Rose Pink                                              |
| 2. Carotin                                               | Deep Blue                                              |
| 3. Quinone                                               | No Color                                               |
| 4. Chloranil                                             | " "                                                    |
| 5. Chlorbutadiene                                        | " "                                                    |
| 6. Tetrahydronaphthalene                                 | " "                                                    |
| 7. Phenol                                                | " "                                                    |
| 8. Acetaldehyde*                                         | " " (No reduction of gold)                             |
| 9. Theelin                                               | " "                                                    |
| 10. Benzal acetophenone                                  | " "                                                    |
| 11. Acetoacetic ester                                    | " "                                                    |
| 12. Allyl chloride                                       | " "                                                    |
| 13. Anthracene                                           | " "                                                    |
| 14. Dianthrane                                           | " "                                                    |
| 15. Chrysene                                             | " "                                                    |
| 16. Phenanthrene                                         | " "                                                    |
| 17. Vinyl acetylene                                      | Deep Yellow                                            |
| 18. 1,3-dibromobutene 2                                  | Orange - pink (definite suggestion of color formation) |
| 19. Isodibrombutene                                      | No Color                                               |
| 20. $\alpha$ -bromostyrene                               | " "                                                    |
| 21. Quinaldine                                           | Yellow Color                                           |
| 22. Fluorene                                             | No Color                                               |
| 23. Crotonaldehyde                                       | Yellow Color                                           |
| 24. Cyclohexene                                          | No Color                                               |
| 25. Cyclohexadiene                                       | No Color                                               |
| 26. 1 methylcyclohexadiene 2,4                           | " "                                                    |
| 27. 1,1 dimethylcyclohexadiene 2,4                       | No Color                                               |
| 28. Eugenol                                              | Yellow Color                                           |
| 29. Cinnamic aldehyde                                    | No Color                                               |
| 30. 9,10 dimethyl 9,10 dihydroxyphenanthrene             | " "                                                    |
| 31. Phenanthraquinone                                    | " "                                                    |
| 32. Morphine                                             | " "                                                    |
| 33. Diamylene                                            | " "                                                    |
| 34. Ascaridol                                            | " "                                                    |
| 35. $\alpha$ -pinene                                     | " "                                                    |
| 36. Borneol chloride                                     | " "                                                    |
| 37. Allyl alcohol                                        | " "                                                    |
| 38. Crotonic acid                                        | " "                                                    |
| 39. Cholesterol (crude, from gall stones)                | " "                                                    |
| 40. Cholesterol (purified by $\text{KMnO}_4$ & recryst.) | " "                                                    |
| 41. Cholesterol (purified by way of dibromide)           | " "                                                    |

\*Acetaldehyde does not show its customary reducing powers in this instance.



HBr isomers of ergosterol, and dehydroergosterol were tested with the auric chloride reagent. The results are summarized in Table I. It is concluded that the secondary alcohol group is not involved in the production of the color. Color production is doubtless bound up with the particular conjugation of double bonds in one of the rings, since the isomers produced by the action of mineral acids and dehydroergosterol do not show the reaction. However, it is not the simple type of conjugation which is found in the dihydrobenzene series, since cyclohexadiene, 1 methylcyclohexadiene 2,4, and 1,1 dimethylcyclohexadiene 2,4 do not produce a color with anhydrous auric chloride.

A similar reagent was prepared by dissolving anhydrous platonic chloride in nitrobenzene and benzonitrile. Platonic chloride was found to be rather insoluble in both of these solvents, a fact which may account for the fainter pink color developed by ergosterol when treated with anhydrous platonic chloride. Carotene, ergosterol and various derivatives of ergosterol were tested. The results are summarized in Table II. Anhydrous platonic chloride as well as auric chloride reacts with ergosterol and those derivatives of ergosterol which involve the hydroxyl group, but does not react with carotin.

### III. The Characteristic Behavior of the Conjugated System of the Ergosterol Molecule

The dehydrogenating effect of mercuric acetate upon the ergosterol molecule is considered to be a reaction of mercuric acetate with the conjugated system of the ergosterol molecule. When an alcoholic solution of ergosterol is treated with mercuric acetate, mercurous acetate is precipitated in quantitative amounts, and the dehydroergosterol may be recovered from the alcoholic filtrate. For instance, when ergosterol is treated with two mols of

mercuric acetate in alcoholic solution at room temperature, two mols of mercurous acetate are formed. With larger quantities of mercuric acetate, the reduction proceeds beyond the two mold stage, due, no doubt, to a secondary reaction. Ergosterol which has been irradiated one and one-half hours shows the same reducing effect upon mercuric acetate (Table IV).

TABLE IV  
REDUCING EFFECT ON MERCURIC ACETATE

| Compound                            | Solvent       | Mols of<br>Hg(Ac) <sub>2</sub> | Time of<br>Reaction | HgAc Formed |               |
|-------------------------------------|---------------|--------------------------------|---------------------|-------------|---------------|
|                                     |               |                                |                     | Wt.<br>gm.  | Per<br>Cent** |
| Ergosterol                          | Ethyl Alcohol | 4                              | 24 hrs.             | .6546       | 125           |
|                                     |               |                                | 48 hrs.             | .7302       | 139           |
|                                     |               |                                | 96 hrs.             | 1.0242      | 195           |
| Ergosterol                          | Glacial HAc   | 4                              | 48 hrs.             | .8577       | 164           |
| Ergosterol                          | Ethyl Alcohol | 3                              | 52 hrs.             | 3.4826      | 133           |
| Ergosterol                          | " "           | 2.2                            | 35 das.             | 5.5063      | 101           |
| Ergosterol                          | " "           | 2                              | 52 hrs.             | 1.1778      | 90            |
| Ergosterol                          | " "           | 2                              | 48 hrs.             | 2.4140      | 92            |
| Ergosterol                          | " "           | 2                              | 36 hrs.             | 2.4800      | 95            |
| Ergosterol                          | " "           | 2                              | 3.5 das.            | 2.5400      | 97            |
| Irradiated (30 min.)<br>Ergosterol  | " "           | 2                              | 48 hrs.             | 1.2102      | 92            |
|                                     |               |                                | 96 hrs.             | 1.2904      | 99            |
| Irradiated (1.5 hrs.)<br>Ergosterol | " "           | 2                              | 12 hrs.             | 1.0962      | 84            |
|                                     |               |                                | 3 das.              | 1.2276      | 94            |
|                                     |               |                                | 5 das.              | 1.2762      | 97            |
| Ergosteryl $\alpha$ -acetate        | " "           | 2                              | 48 hrs.             | 0.1848      | 81            |
| Ergosteryl $\alpha$ -acetate        | " "           | 2                              | 48 hrs.             | 0.1850      | 81            |
| Ergosteryl Benzoate                 | " "           | 2                              | 48 hrs.             | 0.1768      | 88            |
| Ergosteryl $\beta$ -acetate         | " "           | 2                              | 48 hrs.             | 0.1256      | 55            |
| Ergosteryl $\beta$ -acetate         | " "           | 2                              | 48 hrs.             | 0.1008      | 44            |
| Ergosteryl $\beta$ -acetate         | " "           | 2                              | 48 hrs.             | 0.1498      | 65            |
| Ergosteryl $\beta$ -acetate         | " "           | 2                              | 72 hrs.             | 0.1812      | 79            |

\*Percentage calculations on the basis of a two-electron transfer.

In general the reaction of an ethylenic compound with an aqueous mercuric salt consists in the addition of the groups -HgX and -OH to the double bond. Upon continued standing or as a result of the application of heat, the organo-mercury compound may be

oxidized to the glycol. For instance, the organo-mercury compound,  $C_2H_5O-\overset{\overset{H}{|}}{\underset{\underset{H}{|}}{C}}-\overset{\overset{H}{|}}{\underset{\underset{H}{|}}{C}}-HgOAc$ , results from the reaction of ethylene and mercuric acetate in alcoholic solution. This is a general behavior of ethylenic compounds and mercuric salts, and is characteristic also of unsaturated ring compounds such as cyclohexene.

A conjugated system of olefinic double bonds shows the same characteristic behavior with mercuric salts. Chlorobutadiene, an example of this type of compound, adds one mol of mercuric acetate. Conjugated systems of double bonds of the benzenoid type react with mercuric acetate by substitution of the group  $-HgOAc$  for an hydrogen atom in the aromatic molecule. In this manner mercuric acetate reacts with Ar-tetrahydro- $\beta$ -naphthol, Ar-tetrahydro- $\alpha$ -naphthol, but does not react with Ac-tetrahydro- $\beta$ -naphthol, nor with decahydro- $\beta$ -naphthol (Table V).

However, when a conjugated system of the ring type, as, for instance, cyclohexadiene, is treated with mercuric acetate, two simultaneous reactions may take place; i.e., (1) the normal addition of the mercuric acetate as shown by the straight chain aliphatic conjugated systems, as in the case of chlorobutadiene, and (2) the formation of a benzenoid ring. The latter reaction implies the oxidation of a carbon atom and the reduction of mercuric ion to the mercurous state.

Three dihydrobenzenes, 2,4 cyclohexadiene, 1 methylcyclohexadiene 2,4, and 1,4 dimethylcyclohexadiene 2,4 were prepared and treated with mercuric acetate. Cyclohexadiene and methylcyclohexadiene exhibit both types of reaction with mercuric acetate. When treated with 1 mol of mercuric acetate, cyclohexadiene caused the almost immediate precipitation of mercurous acetate. However, the first reaction appeared to be the immediate

addition of mercuric acetate to the unsaturated compound, for the solution showed no test for free mercuric ions after standing 30 minutes. After 5 days, 31 per cent of the calculated amount of mercurous acetate had been precipitated. Continued standing did not increase this amount. Methylcyclohexadiene proved more

TABLE V  
REACTION WITH MERCURIC ACETATE

| Compound                                   | Mols<br>Hg(OAc) <sub>2</sub><br>added | Time<br>Das. | HgOAc<br>Formed<br>% | Carbon-Mercury Compound |             |
|--------------------------------------------|---------------------------------------|--------------|----------------------|-------------------------|-------------|
|                                            |                                       |              |                      | % Mercury               | Calcd. % Hg |
| 1. Chlorobutadiene                         | 0.89                                  | 14           | 0                    | 58.22                   | 58.75       |
| 2. $\alpha$ -pinene                        | 2                                     | 20           | 32.7                 | 0                       | --          |
| 3. Ar-tetrahydro-<br>$\beta$ -naphthol     | 1                                     | 2            | 0                    | 52.60                   | 49.33       |
|                                            | 1                                     | 2            | 0                    | 60.14                   | 49.33       |
|                                            | 2                                     | 2            | 0                    | 60.43                   | 60.30       |
| 4. Ac tetrahydro-<br>$\beta$ -naphthol     | 1                                     | 2            | 0                    | 0                       | 49.33       |
|                                            | 2                                     | 2            | 0                    | 0                       | 60.30       |
| 5. Ar tetrahydro-<br>$\alpha$ -naphthol    | 1                                     | 2            | 0                    | 49.73                   | 49.33       |
|                                            | 1                                     | 2            | 0                    | 59.28                   | 60.30       |
|                                            | 2                                     | 2            | 0                    | 59.31                   | 60.30       |
| 6. Decahydro-<br>$\beta$ -naphthol         | 1                                     | 2            | 0                    | 0                       | --          |
| 7. Cyclohexadiene                          | 1                                     | 2            | 17.5                 | Trace                   | --          |
|                                            | 1                                     | 5            | 31.0                 | 0                       | --          |
|                                            | 1                                     | 21           | 26.8                 | 0                       | --          |
| 8. 1 methyl cyclo-<br>hexadiene 2,4        | 1                                     | 2            | 57.5                 | 0                       | --          |
|                                            | 1                                     | 21           | 47.5                 | 0                       | --          |
| 9. 1,1 dimethyl<br>cyclohexa-<br>diene 2,4 | 1                                     | 21           | 15.6                 | 55.72                   | 55.55       |
| 10. Cholesterol                            | 0.9                                   | 21           | 8.1                  | 0                       | --          |
| 11. Ergosterol                             | 2                                     | 2            | 95.0                 | 0                       | --          |
| 12. HCl isomer of<br>Ergosterol            | 2                                     | 5            | 65.1                 | 0                       | --          |

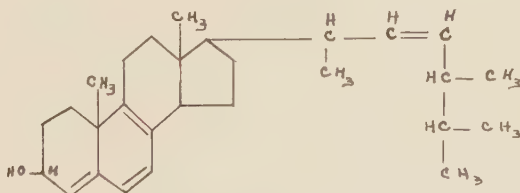
reactive; 58 per cent of the calculated amount of mercurous acetate was recovered. The addition of a saturated solution of sodium chloride caused the precipitation of no organo-mercury compound. In the case of dimethylcyclohexadiene, there was no immediate



precipitation of mercurous acetate. After 21 days, only 16 per cent of the calculated amount of mercurous acetate had been precipitated. An organo-mercury compound was isolated and analyzed. The presence of the second methyl group in this compound has thus hindered the formation of a benzenoid ring.

Ergosterol contains a conjugated system of double bonds. Ergosterol also reduces mercuric acetate to mercurous acetate, and a fourth double bond is formed in the molecule. No organo-mercury compound is produced. The reaction is immediate and quantitative. A consideration of the reaction of the dihydrobenzenes with mercuric acetate indicates that the most probable explanation of the reaction of ergosterol with mercuric acetate is the formation of the benzenoid ring. The presence of a tertiary methyl group at C<sub>10</sub> in the ergosterol molecule would prevent the formation of such a ring. The above evidence is submitted to support the hypothesis that C<sub>10</sub> is not the position of the second methyl group in the ergosterol molecule.

Honigmann<sup>1</sup> presents another formulation of the ergosterol-mercuric acetate reaction. Since the oxidation of dehydroergosterol produces the same produce as does the oxidation of ergosterol, namely, methylbenzene tetracarboxylic acid, Honigmann suggests that the ring containing the original diene bonds in dehydroergosterol has remained unchanged. He therefore suggests the following structure for dehydroergosterol:




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<sup>1</sup>Honigmann, Ann., 508, 89 (1933-1934).

However, cholesterol does not react with mercuric acetate, a fact which suggests that ring I of the ergosterol molecule would probably not gain the new double bond. Ring III is just as unreactive. It therefore seems most probable that mercuric acetate reacts upon ergosterol with the formation of a benzenoid ring, and that C<sub>10</sub> is not the correct position of the second methyl group in the ergosterol molecule. Further experimental work is necessary to establish conclusively which hypothesis is correct.

## EXPERIMENTAL PART

### Irradiation

All irradiations were carried out in quartz tubes fused to graded seals in order that the tubes could be sealed to a vacuum line and evacuated. An air-cooled, hanging quartz-mercury vapor lamp, mounted above a turn-table which rotated the supports of the tubes, and provided uniform irradiation, was used as the source of ultra-violet light. Activity of the irradiated solutions was determined by feeding the irradiated substances dissolved in peanut oil to rachitic rats for a period of five days. Initial and final degree of rickets was determined by x-ray procedure, which provided permanent records of the experiment, and by the Shipley "Line Test" technique. The ergosterol used in the study was a Mead Johnson product, containing one molecule of water, and melted at  $159-161^{\circ}$ , uncorrected,  $[\alpha]_D^{20} = -132^{\circ}$ .

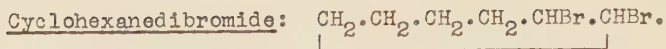
### The Preparation of Cyclohexadiene



A mixture of 123 cc. of cyclohexanol, b. pt.  $160^{\circ}$ , and 4 cc. of concentrated sulfuric acid was heated to  $130-140^{\circ}$ , and the cyclohexene and water were gradually distilled and collected in a chilled receiver. The upper layer containing the cyclohexene was dried over calcium chloride, and redistilled. Yield: 70 gm., 70 per cent of the calculated, b. pt.  $82-86^{\circ}$ .

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<sup>1</sup>Organic Synthesis, I, 175 (1932).



To 70 gm. of cyclohexene in ether solution was added gradually the calculated quantity of bromine, 137 gm., in ether solution. The reaction flask was kept cool, and the reaction was carried out away from direct light. The ether and unchanged cyclohexene were removed by distillation, and the residue was fractionated at 1-2 mm. pressure. Yield: 158 gm., 80 per cent of the calculated,  $N_a^{19,21} = 1.5440$ .



To 1 mol (42 gm.) of cyclohexanedibromide, 3 mols (90 gm.) of freshly distilled quinoline were added, and the mixture was heated under reflux to 190°. The fraction distilling below 95° was recovered, and redistilled over metallic sodium. Yield: 8.3 gm., b. pt. 79-80°, uncorrected.

The compound is a clear, colorless, mobile liquid with a terpene-like odor. The diene produces a cherry red color when it is treated with concentrated sulfuric acid. If acetic anhydride is added, the deep red color changes to a violet blue. Nitric acid causes the production of a blue color. No color results when cyclohexadiene is treated with anhydrous auric chloride reagent.

0.163 gm. + 0.661 gm.  $\text{Hg}(\text{OAc})_2$ : 0.141 gm.  $\text{HgOAc}$ , or 31 per cent of the calculated.

The Preparation of 1 methylcyclohexadiene 2.4



By means of a dropping funnel, 100 gm. of 35 per cent phosphoric acid were added to 100 gm. of 3 methylcyclohexanol,

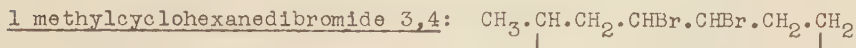
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<sup>1</sup>Crossley, J. Chem. Soc., 85, 1409 (1904).

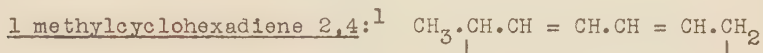
<sup>2</sup>Dehn and Jackson, J. Am. Chem. Soc., 55, 4284 (1933).



b. pt. 173-174°, and the reaction mixture was heated to 160-170°. The distillate was dried and fractionated. Yield: 55 gm., 65 per cent of the calculated, b. pt. 105-106°.



To 55 gm. of 3 methylcyclohexene were added 93 gm. of bromine in ether solution as in the preparation of cyclohexanedibromide. Yield: 95 gm., 64 per cent of the calculated, b. pt. 126-128° at 36 mm. pressure.

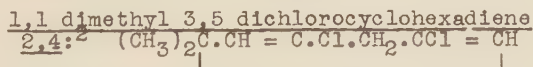


To 54 gm. of 3 methylcyclohexanedibromide 90 gm. of freshly distilled quinoline were added and the reaction mixture was treated as described in the preparation of cyclohexadiene. Yield: 9 gm., b. pt. 105-106°,  $n_D^{21} = 1.4668$ .

The compound is a clear, colorless liquid, with a terpene-like odor, and gives the characteristic color reactions with sulfuric acid, acetic anhydride, and nitric acid. It gives no color reaction when treated with anhydrous auric chloride.

0.66 gm. + 0.563 gm.  $\text{Hg}(\text{OAc})_2$ , 1 mol: 0.269 gm.  $\text{HgOAc}$ , or 57 per cent of the calculated.

The Preparation of 1,1 dimethylcyclohexadiene 2,4

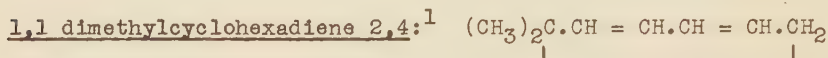


To 50 gm. of dimethyldichlororesorcinol dissolved in 150 gm. of dry chloroform, 150 gm. (2 mols) of phosphorus pentachloride were added gradually, and the reaction mixture was heated gently for 2 hours. The chloroform was removed by evaporation, the

<sup>1</sup>Zelinsky and Gorsky, Ber., 41, 2479 (1908).

<sup>2</sup>Crossley and LeSueur, J. Chem. Soc., 81, 821 (1902).

reaction products poured into water, and the oil-water mixture extracted with ether. The ethereal solution was washed with alkali until neutral, dried over calcium chloride, and fractionated. Yield: 50 gm., 79 per cent of the calculated, b. pt.  $116^{\circ}$  at 66-68 mm. pressure.



All attempts to prepare 1,1 dimethylcyclohexadiene by the action of quinoline upon the hexanedichloride were unsuccessful. A sodium-moist ether reduction was finally used.

To 16 gm. of dimethyldichlorocyclohexadiene in 350 cc. of moist ether were added 14 gm. of metallic sodium, cut in thin slices. The operation required 6 to 7 hours. The solution was heated towards the end of the operation, and small quantities of water were added. The ethereal solution was then poured into water, the immiscible layer separated, and washed until neutral, dried over calcium chloride, and fractionated. The fraction distilling below  $115^{\circ}$  was saved and refractionated. The higher boiling fraction was again treated with sodium. Yield from 50 gm. dichloride: 6.6 gm., b. pt.  $110-111^{\circ}$ ,  $n_D^{21} = 1.4635, 1.4661$ .

The compound is a colorless liquid with a terpene-like odor which is characteristic of the series. It gives a cherry red color with sulfuric acid; it is attacked violently by nitric acid, without color formation. It shows no color reaction with anhydrous auric chloride.

0.163 gm. + 0.49 gm.  $\text{Hg}(\text{OAc})_2$ , 1 mol: 0.066 gm.  $\text{HgOAc}$ , 15.6 per cent of the calculated. After the removal of the mercurous acetate, the filtrate was treated with a solution saturated with sodium chloride. A carbon-mercury compound, weighing

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<sup>1</sup>Crossley and LeSueur, J. Chem. Soc., 81, 821 (1902).

0.108 gm. was precipitated.

Anal.<sup>1</sup> Calcd. for  $C_8H_{13}OHgCl$ : Hg, 55.55.

Found: Hg, 55.72.

Preparation of Ergosteryl  $\beta$ -Acetate:<sup>2</sup>  $C_{28}H_{43}O\overset{O}{\underset{||}{C}}.CH_3$

A solution containing 1 gm. of ergosterol and 10 cc. of acetic anhydride was refluxed for 1 hour. The solution was then poured into water, extracted with ether, and the residue, after the removal of the ether, was recrystallized three times from absolute alcohol. Yield: 0.660 gm., or 60 per cent of the calculated, m. pt. 170-171°, m. pt. of the ergosterol-ergosteryl acetate mixture, 137-138°;  $[\alpha]_D^{19} = -90.4^\circ$ ,  $c = 1$ , in chloroform;  $[\alpha]_D^{19} 5461 = -116.6^\circ$ .

A very minute quantity of the acetate, 0.01 mgm., gives a rose color with anhydrous auric chloride. A solution irradiated with ultra-violet light for 15 minutes shows no antirachitic potency in doses of 1%. Doses of 10% of the irradiated compound, whether hydrolyzed or as the acetate, showed healing when fed to rachitic rats.

0.200 gm. acetate + 0.280 gm. (2 mols)  $Hg(OAc)_2$ :

|          |                                          |
|----------|------------------------------------------|
|          | 0.1008 gm. $HgOAc$ or 44 per cent calcd. |
|          | 0.1256 gm. $HgOAc$ or 55 per cent "      |
| (heated) | 0.1812 gm. $HgOAc$ or 79 per cent "      |

Preparation of Ergosteryl  $\alpha$ -Acetate:  $C_{28}H_{43}O\overset{O}{\underset{||}{C}}.CH_3$

A solution of 2 gm. of ergosterol and 5 gm. of anhydrous, fused potassium acetate in 200 cc. of glacial acetic acid were heated at 100° in an atmosphere of carbon dioxide for 6 hours. The reaction mixture was poured into water, and the product extracted with ether. The residue, after the removal of the ether,

<sup>1</sup>Whitmore, J. Am. Chem. Soc., 55, 1131 (1933).

<sup>2</sup>Heilbron, Sexton, and Spring, J. Chem. Soc., 1929, 926.

was recrystallized three times from absolute alcohol. Yield: 0.909 gm., 41 per cent of the calculated, m. pt. 131-133°; m. pt. of the ergosterol-ergosteryl acetate mixture, 147-149°;  $[\alpha]_D^{25} 5461 = -138.8^\circ$ .

A trace of ergosteryl  $\alpha$ -acetate, 0.01 mgm., produces a color with anhydrous auric chloride. After irradiation of 4 hours duration, 1 mgm. of the acetate produces a color with anhydrous auric chloride which fades immediately. The irradiated acetate induces healing in rachitic rats in doses of 1  $\gamma$ .

0.200 gm. + 0.280 gm.  $\text{Hg}(\text{OAc})_2$ : 0.1850 gm.  $\text{HgOAc}$ , or 80.5 per cent of the calculated.

The Preparation of Ergosteryl Benzoate:<sup>1</sup>  $\text{C}_{28}\text{H}_{43}\text{O} \cdot \overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{C}_6\text{H}_5$

To 2.000 gm. of ergosterol in 40 cc. of dry pyridine were added 0.65 cc. of benzoyl chloride, with constant stirring. The reaction mixture was allowed to stand 30 minutes in an ice bath. The solid was removed by filtration, and recrystallized three times from methyl alcohol-ethyl acetate mixtures. Yield: 0.880 gm., m. pt. 164-165°; m. pt. of the ergosterol-ergosteryl benzoate mixture, 154-155°.

A trace of ergosteryl benzoate, 0.01 mgm., gives a color with anhydrous auric chloride.

0.200 gm. + 0.247 gm.  $\text{Hg}(\text{OAc})_2$ : 0.179 gm.  $\text{HgOAc}$ , or 88 per cent of the calculated.

The Preparation of Ergosteryl Phenyl

Urethane:<sup>2</sup>  $\text{C}_{28}\text{H}_{43}\text{O} \cdot \overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{NHC}_6\text{H}_5$

A mixture of 1.5 gm. of ergosterol and 3 gm. of phenyl-isocyanate in 20 cc. of benzene was refluxed for 3 hours. The

<sup>1</sup> de Zee, Bull. acad. roy. med. Belg., 10, 336 (1930).

<sup>2</sup> Windaus, Z. physiol. Chem., 197, 167 (1931).

benzene was removed, water was added to destroy the unchanged isocyanate, and the urethane was extracted with low-boiling petroleum ether. The residue, after the removal of the ether, was recrystallized three times from ethyl acetate. Yield: 0.838 gm., m. pt. 181-182°,  $[\alpha]_D^{19} = -61.5^\circ$ . A trace of the phenylurethane, 0.01 mgm., produces a pink color when treated with anhydrous auric chloride. After an irradiation period of 4 hours, 1 mgm. of the urethane gives a reddish brown color with anhydrous auric chloride which fades immediately. After 15 minutes irradiation, the biological activity of the urethane in doses of 1  $\gamma$  is nil.

The Preparation of Ergosteryl Methyl Ether:<sup>1</sup>  $C_{28}H_{43}O.CH_3$

To 2.000 gm. of ergosterol in 15 cc. of benzene were added 0.5 gm. of potassium emulsified in 15 cc. of benzene. The mixture was stirred for 2 hours. The calculated amount of methyl iodide, 8 cc., was added, and the whole was refluxed for 3 hours. The cold mixture was diluted with an equal volume of ether, and shaken with water. The ether-benzene solution was separated, and the solvents removed by evaporation. The residue was recrystallized three times from ethyl acetate-ethyl alcohol mixtures. Yield: 0.892 gm., m. pt. 151-152°, m. pt. of the ergosterol-methoxyergostatriene mixture, 139-140°. The solution in chloroform was slightly colored; no specific rotation was determined. The compound showed a negative Beilstein test. A trace of the ether, 0.01 mgm., produces a pink color when treated with anhydrous auric chloride. After an irradiation period of 4 hours, 1 mgm. of the ether gives a reddish brown color with anhydrous auric chloride which fades at once. The biological activity of the irradiated ether in doses of 1  $\gamma$  is nil.

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<sup>1</sup>Heilbron and Simpson, J. Chem. Soc., 1932, 268.



The Preparation of the HBr Isomer of Ergosterol:<sup>1</sup>  $C_{28}H_{44}O$

A mixture of 1.000 gm. of ergosterol in 35 cc. of chloroform and 2 cc. of HBr (sp. gr. 1.39) was shaken in a shaking machine in the dark for 2 hours. The chloroform layer was evaporated and the residue, after washing with 96 per cent ethyl alcohol, was dissolved in 20 cc. of hot ethyl alcohol; the crystals were recrystallized three times from hot alcohol. Yield: 0.262 gm., m. pt.  $134^{\circ}$ ,  $[\alpha]_{D}^{24} 5461 = -44.8^{\circ}$ . The crystals show no Beilstein test. The isomer shows no color reaction with anhydrous auric chloride, and is biologically inactive in doses of  $10\gamma$ .

The Preparation of the HCl Isomer of Ergosterol:<sup>2</sup>  $C_{28}H_{44}O$

A mixture of 1.000 gm. of ergosterol in 35 cc. of chloroform and 2 cc. of HCl (sp. gr. 1.19) was shaken in a shaking machine for 4 hours, and treated as the HBr isomer. Yield: 0.550 gm., m. pt.  $138^{\circ}$ ,  $[\alpha]_{D}^{23} 5461 = -37.1^{\circ}$ , (the solution in chloroform was slightly yellow). The isomer shows no color reaction with anhydrous auric chloride, and is biologically inactive in doses of  $10\gamma$ .

0.2000 gm. + 0.321 gm.  $Hg(OAc)_2$ : 0.170 gm.  $HgOAc$ , or 65 per cent of the calculated.

The Preparation of Dehydroergosterol:<sup>3</sup>  $C_{28}H_{42}O$

A solution of 2.000 gm. of ergosterol and 5.057 gm.  $Hg(OAc)_2$ , 3 mols, and a few drops of glacial acetic acid in 350 cc. of ethyl alcohol was warmed gently and allowed to stand 48 hours. The alcohol was removed in vacuo at  $37^{\circ}$  and the filtrate concentrated to 10 cc. A little water was added, and the

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<sup>1</sup>Bills, J. Biol. Chem., 84, 455 (1929).

<sup>2</sup>Ibid.

<sup>3</sup>Windaus, Ann., 474, 185 (1928).

precipitated solid was filtered, washed with cold alcohol, and recrystallized three times from an ethyl acetate-methyl alcohol mixture. Yield: 0.408 gm., or 22 per cent of the calculated, m. pt.  $146^{\circ}$ ,  $[\alpha]_D^{23} = -149^{\circ}$ ,  $c = 0.24$  gm. in  $\text{CHCl}_3$ . A solution containing 1 mgm. of dehydroergosterol per cc. of benzene gives no color reaction when treated with anhydrous auric chloride. The compound is biologically inactive in doses of  $10\gamma$ .

0.1000 gm. + 0.065 gm.  $\text{Hg}(\text{OAc})_2$ : 0.044 gm.  $\text{HgOAc}$  or 33 per cent of the calculated.

#### The Reaction of Chlorobutadiene and Mercuric Acetate

To 0.94 gm. of chlorobutadiene, b. pt.  $59-60^{\circ}$ , in ethyl alcohol, 2.923 gm. of mercuric acetate (0.89 mols) were added. Mercurous acetate, 0.250 gm., was precipitated. A yellowish-white powder was recovered from the filtrate upon the addition of a saturated solution of sodium chloride.

Anal. Calcd. for  $\text{C}_4\text{H}_6\text{OHgCl}_2$ : Hg, 58.75.

Found: Hg, 58.22.

#### The Preparation of Solutions Containing Vitamin $\text{D}_2$

Ergosterol, 10 mgm., in 5 cc. of absolute alcohol was irradiated by ultra-violet light for 15 minutes. The solution was transferred to a 15 cc. centrifuge tube and a solution containing 30 mgm. of digitonin in 96 per cent ethyl alcohol was added. A small volume of water was added, and the precipitate of unchanged ergosterol was allowed to stand 18 hours in the refrigerator.<sup>1</sup> The precipitated digitonide was filtered from the solution, and weighed: 0.0265 gm. of the digitonide or 69 per cent of the original ergosterol was precipitated. Thus, approximately 31 per cent of the original ergosterol has been converted by irradiation.

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<sup>1</sup>Kofler and Raum, Biochem. Z., 219, 335 (1930).

into a compound giving a soluble digitonide.

The filtrate was evaporated to dryness in a vacuum desiccator, dissolved in 3 cc. of benzene, and treated with anhydrous auric chloride reagent. No color developed. Biological tests with 1/4 of the benzene solution showed excellent healing, thereby proving the presence of the vitamin in the solution.

Ergosterol, 50 mgm., in absolute alcohol was irradiated 20 minutes, and 150 mgm. of digitonin in 96 per cent alcohol were added. The filtrate recovered after the removal of the precipitated digitonide was treated with dilute aqueous sodium hydroxide with gentle warming to hydrolyze the digitonide. The solution was then extracted with ether, and the ethereal solution was washed with water until neutral, and dried over calcium chloride. After the removal of the ether, the residue was dissolved in 3 cc. of anhydrous benzene. This benzene solution showed no color when tested with anhydrous auric chloride reagent. Biological tests indicated good healing. No sample of crystalline vitamin D<sub>2</sub> could be obtained to test further this non-reactivity with auric chloride.

## SUMMARY

- I. The study of the color reaction of ergosterol and anhydrous auric chloride led to the following results:
1. Benzene solutions of ergosterol and of those derivatives of ergosterol involving the secondary alcohol group, the acetates, the benzoate, the phenyl urethane, and the methyl ether, react with anhydrous auric chloride in nitrobenzene or benzonitrile with the production of a rose pink color.
  2. The test is sensitive to 0.005 mgm. of ergosterol.
  3. When solutions of ergosterol and derivatives of ergosterol are treated with auric chloride after a period of prolonged irradiation, an immediate fading of the rose color is produced. Ultra-violet light, therefore, has increased the reducing power of the molecule.
  4. This increased reducing power of ergosterol resulting from the irradiation by ultra-violet light is independent of the presence or absence of oxygen.
  5. Those isomers of ergosterol which result from a change in the internal structure of the molecule, as in the case of the isomerization by means of mineral acids, do not give a color reaction with anhydrous auric chloride. The product of the dehydrogenating action of mercuric acetate upon ergosterol does not produce a color with anhydrous auric chloride.
  6. Evidence is given suggesting that vitamin D<sub>2</sub> does not

give a color reaction with anhydrous auric chloride.

7. Carotin and ergosterol are the only substances among the compounds tested which are found to give a color reaction with auric chloride.
8. Solutions of ergosterol and anhydrous platinic chloride react with color formation.

II. In the study of the reaction of ergosterol with mercuric acetate, the following facts are evident:

1. At room temperature ergosterol reduces 2 mols of mercuric acetate quantitatively to mercurous acetate.
2. Irradiated ergosterol also reduced 2 mols of mercuric acetate quantitatively to mercurous acetate.
3. Conjugated systems of double bonds as found in molecules of the type of chlorobutadiene form organo-mercury compounds by the addition of the salt to the double bond. The conjugated system of double bonds found in the tetrahydronaphthols also form organo-mercury derivatives.
4. Conjugated cyclic systems, such as the dihydrobenzenes, cyclohexadiene and methylcyclohexadiene, reduce mercuric acetate to mercurous acetate. A benzenoid ring is produced in the molecule.
5. 1,1 dimethylcyclohexadiene causes little if any reduction of mercuric acetate to mercurous acetate. An organo-mercury compound was isolated. The formation of a benzenoid ring is impossible with molecules of this type.
6. The formation of a benzenoid ring is postulated as the most logical explanation of the reaction of ergosterol



with mercuric acetate. The reactions of the dihydrobenzenes are submitted as evidence that there is no methyl group at C<sub>10</sub> of the ergosterol molecule.

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